Probing the Active Sites of Monoamine Oxidase A and B with 1,4-Disubstituted Tetrahydropyridine Substrates and Inactivators

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Received February 5, 1997[⊗]

As part of our efforts to characterize more fully the structural features of the monoamine oxidase (MAO) A and B active sites, we have examined the substrate and inhibitor properties of several 1-methyl- and 1-cyclopropyl-4-aryl-1,2,3,6-tetrahydropyridine derivatives with the human placental A and beef liver B forms of the enzyme. We find that the 4-(2-phenylphenyl) analog **23** exhibits a high activity and selectivity for MAO-A while the 4-(3-phenylphenyl) analog **22** shows activity only with MAO-B. Selectivities similar to those of the *N*-methyl series are observed with a series of *N*-cyclopropyl mechanism based inactivators. These results support a topological analysis which attempts to identify steric factors related to the reported substrate and inhibitor selectivities of these two flavoproteins and provide a better definition of the size of the active sites of the two enzymes.

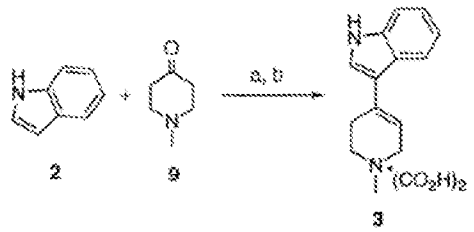
Introduction

The mitochondrial membrane bound flavoenzymes monoamine oxidase A and B (MAO-A and MAO-B) catalyze the α-carbon oxidation of a variety of amines including neurotransmitters such as dopamine and serotonin.[1−3] Although the primary structures of these enzymes have been established from the corresponding gene sequences,[4,5] relatively little is known regarding the structural features of the active sites which lead to the selectivities observed with various substrates[6] and inhibitors.[7−9] Understanding these parameters will lead to better structural insights that could help in identifying potential protoxins and in the design of inhibitors that will be selective for MAO-A and for MAO-B. The excellent MAO-A and/or B substrate and inhibitor properties of various 1,4-disubstituted 1,2,3,6-tetrahydropyridine derivatives offer an interesting opportunity to probe the active sites of these enzymes.[10−12] Studies on the interactions of these tetrahydropyridines with MAO also are prompted by the MAO-catalyzed bioactivation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**1**)] and some of its analogs to generate neurotoxic metabolites.[13,14]

Although a variety of lipophilic C4 substituents are tolerated,[15,16] other modifications, such as reduction of the β,γ-double bond,[17] increase in the size of the *N*-substituent,[18] or introduction of a substituent at any position other than C-4,[19] dramatically decrease the activity.[20,21] The MAO-A vs MAO-B selectivity also is dependent on the nature of the C4 substituent. For example, the V_{max}/K_m ratio for the oxidation of MPTP favors MAO-B by a factor of almost 4[22] while the corresponding ratio for the oxidation of the 2-isopropyl-phenyl analog is 20 in favor of MAO-A.[23]

The results obtained from the examination of a series of flexible 1-methyl-4-arylmethyl and 4-arylethyl MPTP analogs suggest that MAO-A can accommodate bulky substituents more readily than MAO-B.[24,25] Additional data from a systematic structure−substrate activity relationship study employing a series of 1-methyl-1,2,3,6-tetrahydropyridine derivatives bearing a 4-naphthoxy[26] or 4-phenoxy group substituted in the *para*, *meta*, and *ortho* positions with chloro, methoxy, methyl, phenyl, and nitro groups provide supporting evidence for the greater flexibility of the MAO-A active site compared to the active site of MAO-B.[10]

Scheme 1. Synthesis of the 1-Methyl-4-(3-indolyl)-1,2,3,6-tetrahydropyridine Derivative **3**[a]



[a] (a) MeONa, MeOH, reflux, 24 h; (b) $(CO_2H)_2$, Et_2O.

In order to characterize further the spacial features that may contribute to enzyme selectivity, we have determined the K_m and V_{max} values for the MAO-A- and MAO-B-catalyzed oxidations of 1-methyl-1,2,3,6-tetrahydropyridine derivatives bearing various aryl and heteroaryl groups at C4 (see Schemes 1 and 2 for structures). The C4-substituted analogs include the 3-indolyl, the *o*-, *m*-, and *p*-phenyl-substituted phenyl groups, and the previously reported α-naphthyl and β-naphthyl groups.[25] In a parallel series we have examined the k_{inact} and K_I values for the corresponding 1-cyclopropyl analogs which, based on the results of earlier studies, were expected to be mechanism-based inactivators of these enzymes. The data generated from these studies have been used to analyze the relative topology of the MAO-A and -B active sites using computer-assisted molecular modeling. The semiempirical AM1 method was used to generate the minimum energy conformers, and the display software MacMimic (Instar Software, version 91) was utilized to generate the van der Waals volumes of the compounds. Overlay and comparison of these volumes generated images of the topological features of the two active sites.

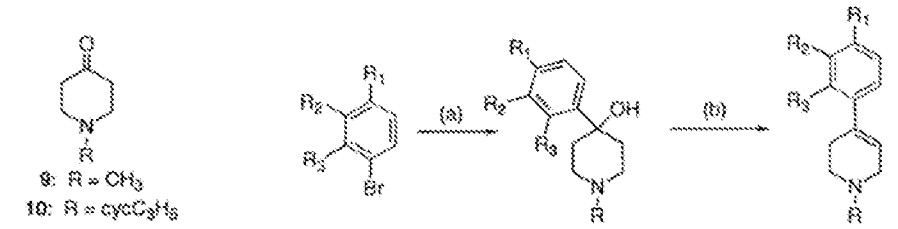
Results and Discussion

Synthesis. The 1-methyl-4-(3-indolyl)-1,2,3,6-tetrahydropyridine (**3**) was prepared in one step by addi-

[⊗] Abstract published in *Advance ACS Abstracts*, May 15, 1997.

S0022-2623(97)00079-4 CCC: $14.00

Scheme 2. Synthesis of the 1-Methyl- and 1-Cyclopropyl-4-aryl-1,2,3,6-tetrahydropyridine Derivatives **21**–**30**



R_1	R_2	R_3		R = CH_3	R = $cycC_3H_5$	R = CH_3	R = $cycC_3H_5$
Ph	H	H	4	11	16	21	26
H	Ph	H	5	12	17	22	27
H	H	Ph	6	13	18	23	28
H	(CH=CH–CH=CH)		7	14	19	24	29
(CH=CH–CH=CH)		H	8	15	20	25	30
H	H	H					31

[a] (a) The bromoarenes **4**–**8** were converted to the Grignard or lithium reagents in THF and then were treated with either **9** or **10** to form the corresponding 4-aryl-4-piperidinols **11**–**20**; (b) heated under reflux for 24 h in AcOH/concentrated HCl (3/1) to give the tetrahydropyridines **21**–**30**, which were characterized as their oxalate salts.

tion of 1-methyl-4-piperidone (**9**) to a solution of indole (**2**) and sodium methoxide in MeOH.[27] The synthetic route to the desired C4-substituted 1-methyl- (**21**–**25**) and 1-cyclopropyl-1,2,3,6-tetrahydropyridines (**26**–**30**) involved addition of the appropriate Grignard or lithiated reagent, derived from the bromoarene (**4**–**8**), to 1-methyl-4-piperidone (**9**) or 1-cyclopropyl-4-piperidone (**10**) to generate the corresponding 4-aryl-4-piperidinols **11**–**15** and **16**–**20**, respectively (Scheme 2). Subsequent acid-catalyzed dehydration provided the desired tetrahydropyridines which were characterized as their stable oxalate salts.

Enzymology. The limited solubility of the test compounds in aqueous solutions required the use of DMSO as cosolvent for the enzyme kinetic studies. We examined the effect of increasing concentrations of DMSO (0–20%) in 100 mM phosphate buffer on the rates of oxidation of 5 mM MPTP and 1 mM 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine by MAO-B and MAO-A, respectively. In the presence of 10% DMSO the MAO-B activity was 90% of the control value and the MAO-A activity was 85% of the control value. At 20% DMSO these values were 60% and 70%, respectively. All of the studies reported here used 10% DMSO.

Estimations of the initial rates of the MAO-A- and MAO-B-catalyzed oxidation of the *N*-methyl analogs **21**–**25** provided linear plots of metabolite concentration vs time. The V_{max} and K_m values were calculated from Lineweaver–Burk double reciprocal plots. A typical example of the Lineweaver–Burk analysis is shown in Figure 1 for the MAO-A-catalyzed oxidation of 1-methyl-4-(2-phenylphenyl)-1,2,3,6-tetrahydropyridine (**23**).

The kinetic parameters obtained in these studies are summarized in Table 1, where the selectivity coefficient ($SC_{A/B}$) represents the V_{max}/K_m ratios for MAO-A/MAO-B. The *p*-biphenyl analog **21** is devoid of both MAO-A and MAO-B substrate properties. This behavior is consistent with the general restriction observed for MAO-B with molecules measuring greater than 12 Å along the N_1–C_4 axis.[28] The *m*-biphenyl analog **22** shows weak substrate properties for MAO-B only. This selectivity for MAO-B is expected on the basis of previous literature data[29,30] with various *meta*-substituted MPTP analogs that showed a loss of MAO-A substrate activity with substituents as small as a methoxy group (Table 2). The 4-(2-phenylphenyl)-, 4-α-naphthyl-, and 4-β-naphthyl-1-methyl-1,2,3,6-tetrahydropyridine analogs (**23**, **24**, and **25**, respectively) proved to be substrates for both MAO-A and MAO-B. In all

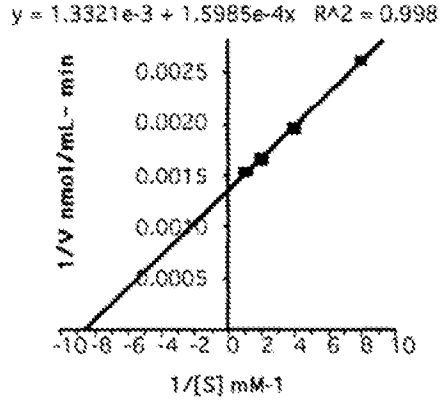


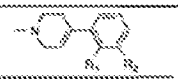
Figure 1. Lineweaver–Burk plot for the MAO-A-catalyzed oxidation of 1-methyl-4-(2-phenylphenyl)-1,2,3,6-tetrahydropyridine (**23**).

Table 1. V_{max} and K_m Values for the MAO-A- and MAO-B-Catalyzed Oxidation of Various 4-Aryl-1-methyl-1,2,3,6-tetrahydropyridine Derivatives

	MAO-A			MAO-B			
compd	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	$SC_{A/B}$[b]
1	20	0.14	143	204	0.39	523	0.27
21		NSD[a]			NSD		–
22		NSD		153	0.97	157	–
23	107	0.12	892	20	1.7	12	74.33
24	526	0.61	862 (1803)	51	0.14	364 (500)	2.37
25	204	0.46	443 (2216)	42	0.59	71 (101)	6.24
3		NSD		358	0.9	397	–

[a] NSD = no substrate properties detected. In parentheses are values reported in the literature.[28] [b] SC = selectivity coefficient.

Table 2. MAO-A and MAO-B Kinetic Data (V_{max}/K_m, min^{-1} mM^{-1}) of Various *m*- and *o*-Phenyl-Substituted MPTP Analogs[21]



	R_1	R_2	MAO-A	MAO-B	$SC_{A/B}$ [b]	$SC_{B/A}$ [b]
1	H	H	144	525	0.27	3.65
32	2-Me	H	588	1288	0.46	2.19
33	2-Et	H	692	296	2.35	0.43
34	2-*i*Pr	H	1122	51	22.0	0.05
35	H	3-Me	76	646	0.12	8.50
36	H	3-OMe	NSO [a]	933	--	--

[a] NSO = no substrate properties detected. [b] SC = Selectivity coefficient.

three cases preference was found for the A form, i.e. $SC_{A/B} > 1$ for these compounds. The greatest MAO-A selectivity is observed with the very bulky *o*-biphenyl analog **23** with $SC_{A/B}$ of 74. This selectivity reflects the very poor MAO-B substrate properties of this compound and is consistent with the trend of enzyme activity and selectivity observed previously with various *ortho*-substituted MPTP derivatives (Table 2). This was interpreted as a consequence of steric restrictions within the active site of MAO-B that are not present in the active site of MAO-A.[16,24,31]

Comparison of our results obtained for the two naphthyl derivatives with those reported in the literature points out the importance of the enzyme source. Particularly impressive are the differences observed here between extracted human placental MAO-A and recombinent MAO-A from human liver expressed in yeast.[25] These differences are unlikely to originate from the methods used because the results obtained with MAO-B, extracted from beef liver in both cases, are very comparable. Due to their accessibility, beef liver MAO-B and human placental MAO-A were used in this and previously reported studies.

None of the *N*-cyclopropyl analogs, **26**−**30**, displayed substrate properties for either form of the enzyme. This is in agreement with the general observation that replacement of the *N*-methyl group of MPTP with a larger substituent leads to loss of good substrate properties.[15,18,28] Additionally, some cyclopropylamines are mechanism-based inactivators of MAO-A and MAO-B,[32,33] and often the enzyme is inactivated too rapidly to allow detectable product formation.[11] On the other hand, we have found that several 4-aryl- and 4-(aryloxy)-1-cyclopropyltetrahydropyridine derivatives are good to excellent MAO-B substrates.[12] The factors which control the partition ratio (number of substrate molecules converted to product per inactivation event) for these compounds remain to be determined.

Several of the 1-cyclopropyltetrahydropyridines prepared in this study were time and concentration dependent inactivators of MAO-A and MAO-B with k_{inact}/K_i values ranging from 0.03 to 1.29 min^{-1} mM^{-1} (Table 3). The selectivities observed with the inhibitor series of the biphenyl analogs **26**−**28** with these enzymes parallel the selectivities of the corresponding substrates. For example, the *p*-biphenyl analog **26** apparently does not have access to the active site of either enzyme since it displayed no inactivator properties, behavior that

Table 3. K_i and k_{inact} Values for the Inactivation of MAO-A and MAO-B by Various 4-Aryl-1-cyclopropyl-1,2,3,6-tetrahydropyridine Derivatives

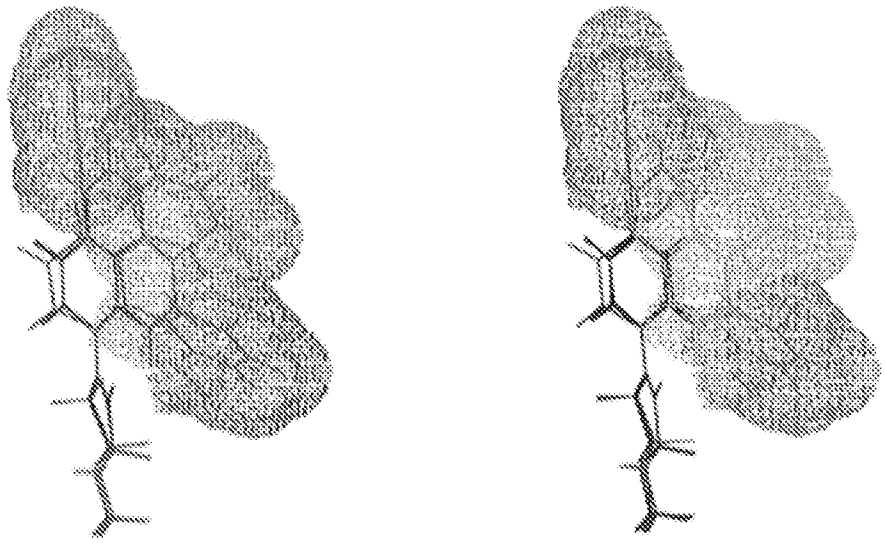
	MAO-A			MAO-B			
compd	k_{inact}	K_i	k_{inact}/K_i	k_{inact}	K_i	k_{inact}/K_i	$SC_{A/B}$ [c]
26		NID [a]			NID		--
27	0.02	0.66	0.03	0.02	0.44	0.05	
28	0.13	0.22	0.59		NID		--
29	0.53	0.40	1.29	0.09	0.11	0.81	1.59
30	0.29	0.38	0.76	0.12	0.13	0.61	1.25
31	0.20	0.05	4.00	0.70	0.18	3.85 [b]	--

[a] NID = no inactivation properties detected. [b] At 37 °C.[33] [c] SC = selectivity coefficient.

parallels that observed with the *N*-methyl analog **21**, which was not a substrate for either enzyme. The *m*-biphenyl analog **27** inhibited MAO-B preferentially while the *N*-methyl analog **22** was a selective MAO-B substrate. The high $SC_{A/B}$ value for the *o*-biphenyl *N*-methyl substrate **23** also is reflected in the MAO-A selective inhibition properties of **28**. The 4-α-naphthyl (**29**) and 4-β-naphthyl (**30**) analogs proved to be effective inactivators of both MAO-A and MAO-B while the *N*-methyl analogs **24** and **25** were substrates for both enzymes although the $SC_{A/B}$ values are higher for the substrates than for the inhibitors. Thus, although the selectivity for MAO-A or MAO-B is conserved in the *N*-methyl series and the *N*-cyclopropyl series, there is no quantitative correlation between substrate and inactivator properties for these series of compounds. In other words, a good substrate will not necessarily lead to an efficient inactivator when the *N*-methyl is replaced by a *N*-cyclopropyl group.

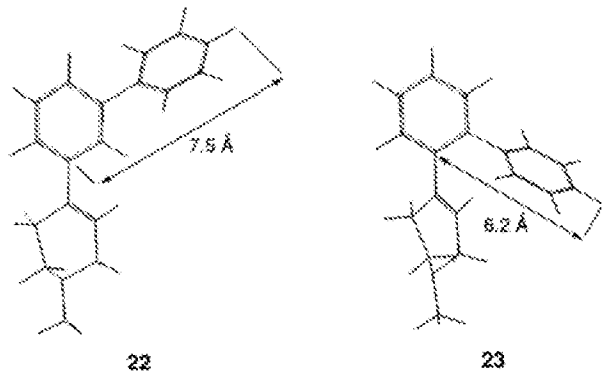
Topological Analysis. We have used the results of these enzyme studies to perform a topological analysis in which we identify features of the active sites of MAO-A and MAO-B. We are not attempting to generate complete models in this preliminary analysis, and therefore compounds from other series were not included in this study. The minimum energy conformers were generated using the semiempirical method AM1.[34−36] The van der Waals volumes about the superimposed minimum energy conformers of these tetrahydropyridine analogs were used to define the regions about the MPTP skeleton that are allowed and disallowed for substrate/inhibitor and nonsubstrate/noninhibitor properties for MAO-A (Figure 2, left panel) and MAO-B (Figure 2, right panel). Analogs that occupy space shown in the blue region in Figure 2, like the *o*-biphenyl analogs **23** and **28**, display enhanced MAO-A activity and selectivity while analogs that occupy space shown in the green region are inactive. Similarly, analogs that occupy space in the pink region shown in Figure 2, like the *m*-biphenyl analogs **22** and **27**, exhibit enhanced MAO-B selectivity while analogs that occupy space shown in the green region are not MAO-B active. The *p*-biphenyl analogs **21** and **26** occupy space that can be accomodated by neither MAO-A nor MAO-B.

As the compounds synthesized in this study are semirigid MPTP type analogs (no flexibility between the carbon C4 and the aryl substituent), the two last biphenyl derivatives can be utilized to obtain new approximations of the size of the active sites. The sizes were measured from the aromatic carbon linked to the C4 position (common atom to all C4 substituents) to the farthest hydrogen of the second phenyl ring (Figure 3).



Left Panel Right Panel

Figure 2. Left panel: MAO-A active (blue) and inactive (green) area. Right panel: MAO-B active (pink) and inactive (green) area.



22 **23**

Figure 3. Minimum sizes of MAO-B and MAO-A defined using biphenyl derivatives **22** and **23**.

The *o*-biphenyl defines a length of 6.2 Å, while the *m*-biphenyl measures 7.5 Å. These results extend substantially the dimensions of the active sites proposed earlier by Efange.[24] Approximate sizes along the same axes in his models were 5.0 Å in the direction of the *meta* substituent for MAO-B and 4.0 Å in the direction of the *ortho* substituent for MAO-A.

The 1-methyl-4-(3-indolyl)-1,2,3,6-tetrahydropyridine derivative **3**, a related analog of the neurotoxin MPTP (**1**), proved to be a good MAO-B substrate, as anticipated by its geometry and spatial features (Figure 4B). As predicted from molecular modeling analysis, this compound, however, was not expected to show MAO-A activity because it points into the region of inactivity defined for MAO-A (Figure 4A—green). Also, a comparison of the dimensions of the indole derivative **3** (5.3 Å) with those of the known MAO-A inactive 3-methoxyphenyl analog **36** (5.4 Å), along the same axis defined above (Figure 5), led to the same prediction, which was confirmed experimentally (Table 1). Thus, although the indole has quite different electronic and polar characteristics from the aryl hydrocarbon derivatives, its MAO-B/MAO-A selectivity can be predicted based on the topology parameters defined with the hydrocarbon series. As we stated previously,[28] this argues that the molecule geometry is an important determinant in defining the activity and selectivity parameters.

A previous study using a series of substituted 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridines revealed that the *meta*-substituted analogs exhibited MAO-A selectivity. In particular, 1-methyl-4-(3-phenylphenoxy)-1,2,3,6-tetrahydropyridine (**37**) shows excellent MAO-A activity (V_{max}/K_m = 5568 min^{-1} mM^{-1}) and the highest MAO-A selectivity of the series ($SC_{A/B}$ = 8.7). Molecular models with compound **37** and the *o*-biphenyl analog **23** show that their minimum energy conformers assume very similar geometries (Figure 6).[37] This analysis provides some rationale for the experimental behavior of the two derivatives.

Conclusion

Using a rather simple topological analysis based on the results obtained with a series of hydrocarbon aryl analogs, the selectivity of various types of C4-substituted tetrahydropyridines derivatives can be explained and predicted. Our results provide additional information that can be used to refine the molecular modeling results reported by Efange *et al.*[23] and the comparative molecular field analyses (CoMFA) developed by Testa and Carrupt[16,21] on 1,4-disubstituted tetrahydropyridine derivatives. More elaborate models of the active sites of both forms of the enzyme, which include all sets of kinetic data available from the literature, are currently being developed.

Experimental Section

Caution: MPTP and other 1,2,3,6-tetrahydropyridines are known or potential nigrostriatal neurotoxins and should be handled using disposable gloves in a properly ventilated hood. Detailed procedures for the safe handling of MPTP derivatives have been reported.[38]

General. Reagents and starting materials were obtained from commercial suppliers and were used without further purification. The syntheses of 1-cyclopropyl-4-piperidone (**10**),[39]

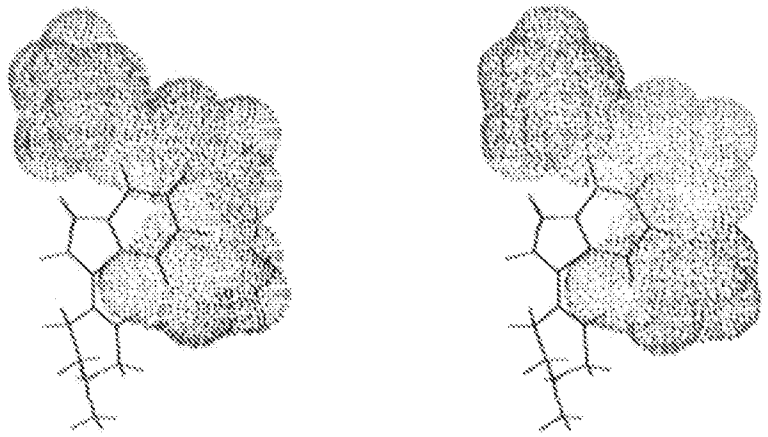


Figure 4. Docking of the 3-indolyl derivative **3** in MAO-A (A, left) and MAO-B (B, right) active sites showing the interaction with the inactive area (green) of MAO-A.

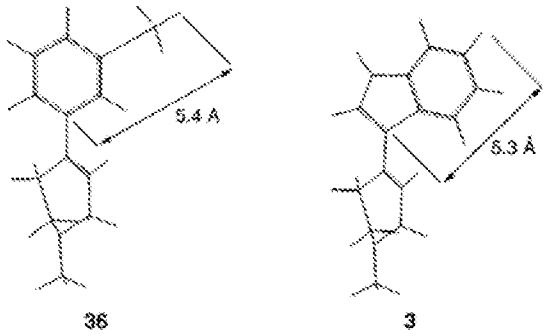


Figure 5. Comparison of the sizes of 3-methoxyphenyl **36** and 3-indolyl **3** derivatives.

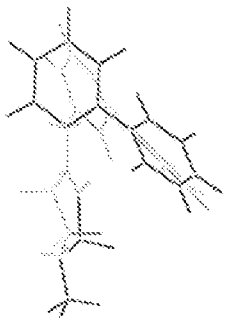


Figure 6. Superimposition of the 4-(3-phenylphenoxy) (light) and 4-(2-phenylphenyl) (dark) derivatives **37** and **23**.

the 4-α- and 4-β-naphthyl-1-methyl-4-piperidinols **14** and **15**, and the corresponding tetrahydropyridines **24** and **25**,[25] respectively, were achieved as reported previously. THF and Et_2O were distilled from sodium/benzophenone ketyl. All reactions were conducted using flame-dried glassware under an atmosphere of dry nitrogen. Chromatography refers to flash column chromatography on silica gel unless otherwise noted. Proton and carbon NMR spectra were recorded on Bruker WP 270-MHz or Varian 400-MHz spectrometers. Exponential function (LB = 0.05−0.2) was applied to the FID to obtain integrals and gaussian function (LB = −1, GB = 0.25) to record coupling constants. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane ($\delta = 0$). Spin multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), or m (multiplet). Coupling constants (*J*) are given in hertz (Hz). Gas chromatography−electron ionization mass spectrometry (GC−EIMS) was performed on a Hewlett-Packard 5890 GC fitted with an HP-1 capillary column which was coupled to a Hewlett-Packard 5870 mass-selective detector. All GC−EIMS were obtained using an initial oven temperature of 60 °C ramping at 25 °C/min to a final temperature of 280 °C with a solvent delay of 2 min and an injection port temperature of 220 °C. Data were processed using an HP 5970 Chemstation. Normalized peak heights are reported as a percentage of the base peak. UV−vis absorption spectra were recorded on a Beckman DU Series 7400 spectrophotometer. Melting points were performed on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA. Calculations and optimizations were performed using the semiempirical AM1 method (Hyperchem),[35] with the restricted Hartree−Fock (RHF) approximation, and the Polack−Ribiere algorythm (gradient fixed at 0.0001) to obtain the lowest energy conformers. Data were saved under PDB file format and imported into MM2-MacMimic (Instar Software, version 91) software.

Chemistry. 1-Methyl-4-(3-indolyl)-1,2,3,6-tetrahydropyridine (**2**),[37] 1-methyl-4-(α-naphthyl)-1,2,3,6-tetrahydropyridine (**24**),[25] and 1-methyl-4-(β-naphthyl)-1,2,3,6-tetrahydropyridine (**25**)[25] were prepared according literature methods, but isolated as their oxalate salts instead of HCl salts. Physical data were consistent with the expected structure for all three compounds.

General Procedure for the Synthesis of 1-Methyl- and 1-Cyclopropyl-4-aryl-4-piperidinols. A solution of 1-methyl-4-piperidone (**9**; 5.8 mmol) or 1-cyclopropyl-4-piperidone (**10**) in 10 mL of THF was added dropwise to a solution of the Grignard (0 °C) or lithium (−78 °C, compound **8** only) reagent derived from the bromoarenes **4**−**8** (5 mmol) in 10 mL of THF. After the reaction mixture was stirred at −78 °C for 30 min and at room temperature overnight, saturated aqueous NH_4Cl (25 mL) was added. The aqueous layer was washed with Et_2O, made to pH 10 with 40% aqueous NaOH, and extracted three times with 25 mL of CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated to yield the crude product which was purified by chromatography on silica gel.

1-Methyl-4-(4-phenylphenyl)-4-piperidinol (11): obtained as a yellow solid from SiO_2 with CH_2Cl_2/2% MeOH (38% yield); mp 159 °C; ^1H NMR ($CDCl_3$) δ 7.56 (6H, m), 7.40 (2H, tt, *J* = 1.4 Hz, *J* = 7.2 Hz), 7.33 (1H, tt, *J* = 2.1 Hz, *J* = 7.2 Hz), 2.75 (2H, bd, *J* = 11.2 Hz), 2.51 (2H, dt, *J* = 2.0 Hz, *J* = 12.4 Hz), 2.35 (3H, s), 2.21 (2H, dt, *J* = 4.8 Hz, *J* = 13.6 Hz), 1.78 (2H, dd, *J* = 2.0 Hz, *J* = 14.0 Hz), 1.70 (1H, s); ^{13}C NMR ($CDCl_3$) δ 140.5, 140.3, 128.8, 127.5, 127.2, 127.0, 124.9, 69.9, 51.4, 44.8, 37.0; GC (t_R 10.39 min)−EIMS *m/z* (rel intensities) 267 (57), 249 (54), 196 (22), 152 (35), 96 (30), 70 (93), 42 (100); UV (MeOH, nm) 220, 253, 320. Anal. ($C_{18}H_{21}NO$) C, H, N.

1-Methyl-4-(3-phenylphenyl)-4-piperidinol (12): obtained as a viscous yellow oil from SiO_2 with AcOEt/2% MeOH (42% yield); ^1H NMR ($CDCl_3$) δ 7.76 (1H, t, *J* = 1.6 Hz), 7.57 (1H, m), 7.43 (7H, m), 2.75 (2H, bd, *J* = 11.0 Hz), 2.47 (2H, dt, *J* = 2.5 Hz, *J* = 12.3 Hz), 2.33 (3H, s), 2.23 (2H, dt, *J* = 4.5 Hz, *J* = 13.5 Hz), 2.03 (1H, bs), 1.79 (2H, dd, *J* = 2.5 Hz, *J* = 14.0 Hz); ^{13}C NMR ($CDCl_3$) δ 148.7, 140.8, 128.2, 127.6, 125.5, 123.8, 123.1, 71.2, 49.7, 38.4, 38.1; GC (t_R 10.19 min)−EIMS *m/z* (rel intensities) 267 (48), 249 (26), 165 (11), 152 (26), 115 (4), 96 (20), 70 (100); UV (MeOH, nm) 219, 264, 324. Anal. ($C_{18}H_{21}NO$) C, H, N.

1-Methyl-4-(2-phenylphenyl)-4-piperidinol (13): obtained as a yellow solid recrystallized from CH_2Cl_2/hexane (81% yield): mp 112 °C; ^1H NMR ($CDCl_3$) δ 7.52 (1H, dd, J = 1.2 Hz, J = 8.0 Hz), 7.32 (7H, m), 7.07 (1H, dd, J = 1.4 Hz, J = 7.4 Hz), 2.65 (2H, bd, J = 10.9 Hz), 2.35 (2H, bd, J = 12.4 Hz), 2.28 (3H, s), 2.21 (2H, bd, J = 12.1 Hz), 1.80 (2H, d, J = 10.9 Hz), 1.53 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 144.2, 143.6, 140.3, 132.6, 129.3, 128.2, 127.7, 127.3, 126.5, 126.0, 72.3, 51.2, 45.3, 38.0; GC (t_R 9.41 min)–EIMS *m/z* (rel intensities) 267 (48), 249 (22), 165 (22), 152 (33), 115 (4), 96 (26), 70 (100), 58 (98); UV (MeOH, nm) 216, 260, 319. Anal. ($C_{18}H_{21}NO$) C, H, N.

1-Cyclopropyl-4-(1-naphthyl)-4-piperidinol (19): ^1H NMR ($CDCl_3$) δ 8.82 (1H, m), 7.84 (1H, m), 7.76 (1H, d, J = 10.6 Hz), 7.45 (4H, m), 2.9 (2H, m), 2.7 (2H, m), 2.3 (1H, m), 1.57 (2H, bs), 1.18 (m, 2H), 0.87 (m, 2H); GC (t_R 11.78 min)–EIMS *m/z* (rel intensities) 267 (13), 238 (20), 169 (15), 141 (24), 127 (34), 82 (100), 68 (61). The remaining reaction mixture was evaporated and used as is.

1-Cyclopropyl-4-(2-naphthyl)-4-piperidinol (20): chromatographed on silica gel (Hex/AcOEt, 7:3) to give the expected product (**20**) as a yellowish solid (1.2 g, 4.5 mmol, 52% yield); mp 124–125 °C; ^1H NMR ($CDCl_3$) δ (1H, d, J = 2.0 Hz), 7.84 (3H, m), 7.66 (1H, dd, J = 2.0 Hz, J = 8.7 Hz), 7.47 (2H, m), 3.01 (2H, m, bd like), 2.72 (2H, dt, J = 2.7 Hz, J = 12.4 Hz), 2.22 (2H, dt, J = 4.7 Hz, J = 13.4 Hz), 1.84 (2H, dq, J = 2.7 Hz, J = 12.4 Hz), 1.72 (1H, m), 1.68 (1H, s), 0.46 (4H, m); ^{13}C NMR ($CDCl_3$) δ 133.2, 132.4, 128.2, 128.0, 127.5, 126.1, 125.8, 123.4, 123.0, 71.6, 49.7, 38.7, 38.4, 5.2; GC (t_R 11.78 min)–EIMS *m/z* (rel intensities) 267 (33), 238 (24), 183 (15), 155 (28), 127 (41), 82 (100), 68 (56); UV (MeOH, nm) 212, 228, 267. Anal. ($C_{18}H_{21}NO$) C, H, N.

1-Cyclopropyl-4-(4-phenylphenyl)-4-piperidinol (16): chromatographed on silica gel (CH_2Cl_2, 100%, and then CH_2Cl_2/MeOH, 92:8) to give the 1-cyclopropyl-4-(4-phenylphenyl)-4-piperidinol (**16**) as a white solid (2.1 g, 7.16 mmol, 72% yield); mp 145 °C; ^1H NMR ($CDCl_3$) δ 7,58 (6H, m), 7.42 (2H, tt, J = 1.8 Hz, J = 7.6 Hz), 7.36 (1H, tt, J = 1.8 Hz, J = 7.4 Hz), 2.97 (2H, m, bd like, J = 11.2 Hz), 2.70 (2H, dt, J = 2.6 Hz, J = 12.2 Hz), 2.14 (2H, dt, J = 4.7 Hz, J = 13.3 Hz), 1.78 (2H, dq, J = 2.6 Hz, J = 11.3 Hz), 1.70 (1H, m), 1.65 (1H, bs), 0.48 (4H, m); ^{13}C NMR ($CDCl_3$) δ 147.6, 140.7, 139.8, 128.7, 127.2, 127.0, 125.0, 71.4, 49.6, 38.7, 38.4, 5.9; GC (t_R 12.60 min)–EIMS *m/z* (rel intensities) 293 (60), 264 (39), 209 (16), 181 (39), 152 (48), 112 (30), 97 (47), 82 (100), 68 (58); UV (MeOH, nm) 215, 252. Anal. ($C_{20}H_{23}NO$) C, H, N.

1-Cyclopropyl-4-(3-phenylphenyl)-4-piperidinol (17): syrupy (72% yield); ^1H NMR ($CDCl_3$) δ 7.74 (1H, t, J = 1.8 Hz), 7.56 (1H, dt, J = 1.7 Hz, J = 7.0 Hz), (6H, m), 7.33 (1H, tt, J = 1.4 Hz, J = 7.2 Hz), 2.94 (2H, m, bd like, J = 12.0 Hz), 2.69 (2H, dt, J = 2.7 Hz, J = 12.2 Hz), 2.15 (2H, dt, J = 4.7 Hz, J = 13.5 Hz), 1.78 (2H, dq, J = 2.4 Hz, J = 13.7 Hz), 1.70 (1H, m), 1.66 (1H, bs), 0.46 (4H, m). ^{13}C NMR ($CDCl_3$) δ 149.1, 141.3, 128.7, 127.2, 125.8, 123.6, 123.5, 71.6, 49.6, 38.7, 38.5, 5.9; GC (t_R 12.35 min)–EIMS *m/z* (rel intensities) 293 (65), 264 (42), 209 (10), 181 (19), 152 (35), 112 (17), 97 (57), 82 (100), 68 (54); UV (MeOH, nm) 215, 249. Anal. ($C_{20}H_{23}NO$) C, H, N.

1-Cyclopropyl-4-(2-phenylphenyl)-4-piperidinol (18): white solid (50% yield); mp 97 °C; ^1H NMR ($CDCl_3$) δ 7.50 (1H, dd, J = 1.4 Hz, J = 8.0 Hz), 7.35 (6H, m), 7.23 (1H, dt, J = 1.4 Hz, J = 7.3 Hz), 7.05 (1H, dd, J = 1.6 Hz, J = 7.4 Hz), 2.79 (2H, m, bd like, J = 12.0 Hz), 2.51 (2H, dt, J = 2.7 Hz, J = 12.2 Hz), 2.10 (2H, dt, J = 4.7 Hz, J = 13.2 Hz), 1.78 (2H, dq, J = 2.4 Hz, J = 14.0 Hz), 1.55 (1H, m), 1.49 (1H, s), 0.39 (4H, m); ^{13}C NMR ($CDCl_3$) δ 145.2, 144.0, 140.6, 132.5, 129.4, 128.0, 127.4, 127.1, 126.1, 125.8, 73.4, 49.3, 38.9, 38.4, 5.9; GC (t_R 11.41 min)–EIMS *m/z* (rel intensities) 293 (10), 264 (19), 209 (5), 181 (22), 152 (34), 112 (11), 101 (52), 82 (100), 68 (59); UV (MeOH, nm) 213, 218, 264. Anal. ($C_{20}H_{23}NO$) C, H, N.

General Procedure for the Synthesis of Tetrahydropyridine Oxalate Salts. Piperidinols (2.1 mmol) were dehydrated by stirring with AcOH/HCl, 3:1 (40 mL), at reflux for 24 h. After cooling, the reaction mixture was slowly basified to pH 9 using 40% NaOH and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude extract was purified by silica gel chromatography to yield the pure tetrahydropyridine which was crystallized as its oxalate salt by addition of oxalic acid in Et_2O. The product was recrystallized from the appropriate solvent.

Oxalate salt of 1-methyl-4-(4-phenylphenyl)-1,2,3,6-tetrahydropyridine (21): yellow solid recrystallized from 2-propanol (87% yield): mp 233–234 °C; ^1H NMR (DMSO-d_6) δ 7.68 (4H, m), 7.57 (2H, m), 7.46 (2H, t, J = 7.1 Hz), 7.37 (1H, t, J = 7.2 Hz), 6.26 (1H, s), 3.79 (2H, bs), 3.34 (2H, t, J = 5.9 Hz), 2.88 (3H, s), 2.49 (2H, d, J = 5.9 Hz); ^{13}C NMR (DMSO-d_6) δ 162.1, 139.9, 139.7, 137.9, 133.8, 129.4, 128.1, 127.3, 127.0, 125.8, 118.2, 52.3, 50.4, 42.6, 24.3; GC (t_R 10.12 min)–EIMS *m/z* (rel intensities) 249 (100), 248 (65), 220 (17), 191 (22), 167 (17), 152 (11), 115 (4), 96 (41); UV (nm, MeOH) 230, 283. Anal. ($C_{20}H_{21}NO_4$) C, H, N.

Oxalate salt of 1-methyl-4-(3-phenylphenyl)-1,2,3,6-tetrahydropyridine (22): white solid recrystallized from MeOH/Et_2O (58% yield): mp 192–193 °C; ^1H NMR (DMSO-d_6) δ 7.67 (3H, m), 7.59 (1H, m), 7.44 (5H, m), 6.30 (1H, bs), 3.77 (2H, d, J = 2.7 Hz), 3.33 (2H, t, J = 5.8 Hz), 2.80 (3H, s), 2.49 (2H, m); ^{13}C NMR (CD_3OD) δ 160.6, 140.1, 139.8, 134.4, 131.8,128.9, 128.6, 127.2, 126.7, 126.6, 123.8, 123.6, 115.8, 52.1, 50.7, 41.5, 24.4; GC (t_R 9.94 min)–EIMS *m/z* (rel intensities) 249 (100), 248 (59), 205 (15), 191 (24), 165 (17), 152 (13), 115 (5), 96 (57); UV (nm, MeOH) 233, 272. Anal. ($C_{20}H_{21}NO_4$) C, H, N.

Oxalate salt of 1-methyl-4-(2-phenylphenyl)-1,2,3,6-tetrahydropyridine (23): white solid recrystallized from MeOH/Et_2O (69% yield): mp 187–188 °C; ^1H NMR (DMSO-d_6) δ 7.35 (9H, m), 5.62 (1H, s), 3.62 (2H, bs), 3.02 (2H, t, J = 5.7 Hz), 2.69 (3H, s), 2.50 (2H, bs); ^{13}C NMR (CD_3OD) δ 163.9, 140.8, 140.2, 139.1, 138.2, 130.1, 128.9, 128.5, 128.3, 128.1, 127.4, 127.1, 119.2, 51.9, 50.3, 41.3, 25.9; GC (t_R 8.83 min)–EIMS *m/z* (rel intensities) 249 (48), 248 (46), 205 (20), 191 (39), 178 (43), 152 (13), 115 (3), 96 (59), 58 (100); UV (nm, MeOH) 237, 270. Anal. ($C_{20}H_{21}NO_4$) C, H, N.

Oxalate Salt of 1-Cyclopropyl-4-(1-naphthyl)-1,2,3,6-tetrahydropyridine (29). The product was recrystallized from MeOH (dehydration 47% yield, recrystallization 75% yield): mp 143–144 °C; ^1H NMR (DMSO-d_6) δ 7.55 (2H, m), 7.46 (1H, d, J = 7.0 Hz), 7.09 (3H, m), 6.91 (1H, d, J = 7.0 Hz), 5.32 (1H, bs), 3.40 (2H, bs), 3.00 (2H, bs), 2.24 (2H, bs), 2.10 (1H, m), 0.52 (2H, m), 0.35 (2H, m); ^{13}C NMR (DMSO-d_6) δ 164.1, 140.3, 135.9, 133.8, 131.0, 128.8, 127.9, 126.7, 126.4, 126.0, 125.7, 125.3, 122.4, 51.6, 50.0, 38.5, 29.5, 4.7; GC (t_R 11.46 min)–EIMS *m/z* (rel intensities) 249 (72), 234 (100), 206 (9), 192 (22), 178 (35), 165 (50), 152 (20), 115 (4), 68 (7); UV (nm, MeOH) 221, 280. Anal. ($C_{20}H_{23}NO_4$) C, H, N.

Oxalate Salt of 1-Cyclopropyl-4-(2-naphthyl)-1,2,3,6-tetrahydropyridine (30). The product was recrystallized from MeOH (dehydration 52% yield, recrystallization 97% yield): mp 192 °C; ^1H NMR (DMSO-d_6) δ 7.80 (4H, m), 7.40 (1H, dd, J = 1.7 Hz, J = 8.7 Hz), 7.19 (2H, m), 6.05 (1H, bs), 3.47 (2H, d, J = 1.1 Hz), 3.03 (2H, t, J = 5.9 Hz), 2.50 (2H, bs), 2.20 (1H, m), 0.52 (2H, m), 0.40 (2H, m); ^{13}C NMR (DMSO-d_6) δ 163.4, 136.2, 133.8, 133.0, 132.2, 128.1, 127.9, 127.3, 126.3, 123.2, 123.0, 119.3, 51.4, 49.3, 37.7, 25.0, 4.2; GC (t_R 11.50 min)–EIMS *m/z* (rel intensities) 249 (59), 234 (100), 206 (9), 178 (46), 165 (57), 152 (25), 115 (11), 89 (8), 68 (11), 54 (26); UV (nm, MeOH) 241, 247, 274, 282, 297. Anal. ($C_{20}H_{23}NO_4$) C, H, N.

Oxalate salt of 1-cyclopropyl-4-(4-phenylphenyl)-1,2,3,6-tetrahydropyridine (26): white solid (dehydration 99% yield, recrystallization from MeOH 83% yield): mp 213–214 °C dec; ^1H NMR (DMSO-d_6) δ 7.67 (4H, m), 7.54 (2H, m), 7.46 (2H, tt, J = 1.4 Hz, J = 7.1 Hz), 7.36 (1H, tt, J = 1.3 Hz, J = 7.2 Hz), 6.24 (1H, m), 3.63 (2H, d, J = 2.6 Hz), 3.21 (2H, t, J = 5.9 Hz), 2.67 (2H, bs), 2.35 (1H, m), 0.66 (4H, m); ^{13}C NMR (DMSO-d_6) δ 162.7, 139.5, 139.3, 138.0, 133.7, 129.0, 127.6, 126.7, 126.5, 125.3, 118.3, 51.3, 49.4, 37.8, 24.8, 4.2; GC (t_R 12.45 min)–EIMS *m/z* (rel intensities) 275 (48), 260 (100), 203 (18), 191 (35), 178 (31), 165 (30), 152 (20), 115 (18), 68 (35), 54 (47); UV (nm, MeOH) 209, 279. Anal. ($C_{22}H_{23}NO_4$) C, H, N.

Oxalate salt of 1-cyclopropyl-4-(3-phenylphenyl)-1,2,3,6-tetrahydropyridine (27): white solid (dehydration 98% yield, recrystallization from MeOH 67% yield): mp 169–170 °C; ^1H

NMR (DMSO-d_6) δ 7.69 (3H, m), 7.56 (1H, m), 7.48 (4H, m), 7.36 (1H, tt, J = 1.3 Hz, J = 7.2 Hz), 6.27 (1H, m), 3.69 (2H, d, J = 2.7 Hz), 3.23 (2H, t, J = 5.8 Hz), 2.71 (2H, bs), 2.41 (1H, m), 0.72 (2H, m), 0.66 (2H, m); ^{13}C NMR (DMSO-d_6) δ 163.6, 140.5, 140.1, 139.9, 134.1,129.1, 128.9, 127.6, 126.8, 126.0, 123.9, 123.2, 118.9, 51.3, 49.4, 37.7, 25.0, 4.2; GC (t_R 12.06 min)−EIMS m/z (rel intensities) 275 (48), 260 (100), 203 (20), 191 (31), 178 (34), 165 (40), 152 (25), 115 (20), 77 (23), 68 (64), 54 (76); UV (nm, MeOH) 205, 209, 245. Anal. ($C_{23}H_{25}NO_4$) C, H, N.

Oxalate salt of 1-cyclopropyl-4-(2-phenylphenyl)-1,2,3,6-tetrahydropyridine (28): white solid (dehydration 99% yield, recrystallization from MeOH 83% yield); mp 185 °C; ^{1}H NMR (DMSO-d_6) δ 7.35 (8H, m), 7.24 (1H, dd, J = 2.2 Hz, J = 6.8 Hz), 5.62 (1H, bs), 3.52 (2H, d, J = 2.2 Hz), 2.89 (2H, t, J = 5.7 Hz), 2.32 (1H, m), 2.02 (2H, bs), 0.65 (2H, m), 0.59 (2H, m); ^{13}C NMR (DMSO-d_6) δ 163.7, 141.1, 140.2, 139.2, 136.9, 130.0, 129.1, 128.5, 128.4, 127.8, 127.5, 127.1, 121.8, 51.1, 49.0, 37.5, 26.8, 4.1; GC (t_R 10.69 min)−EIMS m/z (rel intensities) 275 (68), 260 (100), 203 (30), 191 (60), 178 (51), 165 (51), 152 (19), 115 (15), 82 (43), 68 (42), 54 (60); UV (nm, MeOH) 208. Anal. ($C_{23}H_{25}NO_4$) C, H, N.

Enzyme Studies. The isolation and purification of MAO-A from human placenta and MAO-B from beef liver were carried out using the procedures reported by Salach[30] with the following changes. The phospholipase A used in the preparation was obtained commercially from Sigma. We did not subject the MAO-A preparation to the Sephadex purification or the MAO-B preparation to the glucose gradient purification step. In both cases, however, we obtained highly active preparations.

In preliminary studies, solutions of the oxalate salts of the 1-methyl-4-substituted-1,2,3,6-tetrahydropyridine analogs in phosphate buffer (pH = 7.4, 0.5 mM) were treated with 20 μL of MAO-A (final concentration 0.16 μM) or 5 μL of MAO-B (final concentration 0.16 μM), and the substrate properties were evaluated at 30 °C. The qualitative substrate properties were obtained from a series of UV scans (500−250 nm) vs time over a 1 h period for each compound.

Kinetic Studies. Solutions of the test compounds [final volume of 500 μL, final substrate concentration (100−2000 μM)] in 10% DMSO sodium phosphate buffer (100 mM, pH = 7.4) were incubated in the presence of 0.16 μM MAO-A or 0.08 μM MAO-B. The rates of oxidation were obtained by monitoring the increment in the dihydropyridinium absorbance every 3 s over a 2 min time period. In the case of the 4-α-naphthyltetrahydropyridine analog (**14**), the λ value monitored was 316 nm, and for the 4-β-naphthyl analog (**15**), λ = 328 nm was monitored. The dihydropyridinium products of the C4 biphenyl analogs **16**−**18** appear at 322, 340, and 325 nm, respectively. The K_m and V_{max} values were calculated from Lineweaver−Burk double-reciprocal plots.

The inactivation studies on the 1-cyclopropyl-4-substituted-1,2,3,6-tetrahydropyridine analogs were conducted as follows: Standard solutions of the test compounds (ranging from 2000 to 500 μM) in 10% DMSO sodium phosphate buffer (100 mM, pH = 7.4) were prepared. Each solution (50 μL) was mixed with 50 μL of 0.16 μM MAO-A or 0.08 μM MAO-B, and the resulting mixture was incubated in a 30 °C water bath. A 10 μL aliquot of each incubation mixture was taken at 0, 5, 10, 15, and 20 min and added to a sample cuvette containing 490 μL of 1 mM solution of 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine in sodium phosphate (pH = 7.4, 100 mM) for the MAO-A studies or 490 μL of a 5 mM solution of MPTP in sodium phosphate (pH = 7.4, 100 mM) for the MAO-B studies. The rate of oxidation of 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine was determined at 30 °C by monitoring the formation of amine enone at λ = 324 nm,[31] and the rate of oxidation of MPTP was obtained by monitoring the increment in the dihydropyridinium metabolite λ = 343 nm every 3 s for 2 min.

Acknowledgment. This study was supported by the National Institute of Neurological and Communicative Disorders and Stroke (NS 28792), the Harvey W. Peters Center for the Study of Parkinson's Disease, and a Lavoisier Fellowship to S. Mabic.

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JM970079R